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First country record of the fanged frog *Limnonectes nguyenorum* McLeod, Kurlbaum & Hoang, 2015 (Anura, Dicroglossidae) in China

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http://zoobank.org/709A11C3-AC4F-4543-9CB8-7B7A2C3C632E

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Abstract

We report the first record of *Limnonectes nguyenorum* McLeod, Kurlbaum & Hoang, 2015 outside of Vietnam, namely from China, based on five specimens collected from Daweishan Nature Reserve, southern Yunnan, China. Morphologically, the records from China agree with those of *L. nguyenorum* from Vietnam, and they also phylogenetically clustered with strong support. In addition, based on the new records from China and the previous descriptions of *L. nguyenorum* from Vietnam, we provide an extended diagnosis of this species.

Key Words

16S rRNA, Daweishan Nature Reserve, Honghe Prefecture, morphology, southern Yunnan

Introduction

The fanged frog genus *Limnonectes* Fitzinger, 1843 is the most species rich genus of Asian frogs of the family Dicroglossidae which presently comprises 78 species distributed throughout East and Southeast Asia (Frost 2021). To date, six species have been reported from China (Wang et al. 2020; AmphibiaChina 2021), namely *L. fragilis* (Liu & Hu, 1973); *L. liui* (Yang, 1983); *L. fujianensis* Ye & Fei, 1994; *L. bannaensis* Ye, Fei & Jiang, 2007; *L. taylori* Matsui, Panha, Khonsue & Kuraishi, 2010; and *L. longchuanensis* Suwannapoom, Yuan, Chen, Sullivan & McLeod, 2016.

Limnonectes nguyenorum McLeod, Kurlbaum & Hoang, 2015 is a species which was described from Ha Giang Province, Vietnam, and then was recorded from Vinh Phuc (Ziegler et al. 2015), Son La (Pham et al. 2016), Lao Cai (Nguyen et al. 2019), Yen Bai (Le and Do 2019), and Tuyen Quang (Pham et al. 2019) provinces. At the time, this species was still believed to be endemic to northern Vietnam.

During our field survey in southern Yunnan, China, in 2019, some specimens of *Limnonectes* were collected from Daweishan Nature Reserve. Detailed morphological comparisons and molecular analyses indicated these specimens to be *L. nguyenorum*. Herein, we provide the first country record for China.

Materials and methods

The field survey was conducted in Daweishan Nature Reserve under the permit of Honghe Prefecture Forestry and Grassland Bureau of Yunnan Province. Specimens were euthanized and then fixed in 75% ethanol for storage after taking photographs. Liver tissue samples were preserved

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in 99% ethanol for molecular analysis. All specimens were deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Genomic DNA was extracted from liver tissues preserved in 99% ethanol using the standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Partial fragments of the mitochondrial 16S rRNA were amplified for all samples via the polymerase chain reaction (PCR) using the primers 16SAR: 5'-CGCCTGTTTAYCAAAAACAT-3' and 16SBR: 5'-CCGGTYTGAACTCAGATCAYGT-3' (Palumbi et al. 1991). PCR cycling conditions followed Suwannapoom et al. (2021). The products were purified and sequenced by Tsingke Biotechnology (Beijing) Co., Ltd., using the same primers as in PCR. All new sequences were deposited in GenBank. *Fejervarya limnocharis* (Gravenhorst, 1829) and *F. iskandari* Veith, Kosuch, Ohler & Dubois, 2001 were chosen as outgroups according to Suwannapoom et al. (2021). Homologous and outgroup sequences were obtained from GenBank (Table 1). The technical computation methods for the sequences alignment, best substitution model selection, Bayesian inference and Maximum likelihood phylogenetic analyses were the same as those in Liu et al. (2021).

Measurements were taken with a digital caliper to the nearest 0.1 mm. Morphological terminology followed McLeod et al. (2015). Abbreviations of the morphometric traits are as follows: **ED**, eye diameter; **EN**, eye–nostril distance; **FEL**, thigh (femur) length; **FOL**, foot length; **HL**, head length; **HW**, head width; **IN**, internarial distance; **IO**, interorbital width; **LAL**, lower arm length; **OH**, odontoid height; **PAL**, palm length; **RFL**, relative finger length when digits are adpressed; **RL**, rostrum length, distance from the level of the anterior corner of the eye to the anteriormost point of the head; **RTL**, relative toe length when digits are adpressed; **SVL**, snout–vent length; **TBL**, shank (tibia) length; **TD**, tympanum diameter; and **UEW**, upper eyelid width.

Results

The obtained sequence alignment is 900 bp in length. Bayesian Inference and Maximum Likelihood analyses showed consistent topology. The specimens collected from Daweishan Nature Reserve, southern Yunnan, China, clustered with the specimens (including type specimens) of *Limnonectes nguyenorum* from Vietnam with strong support by both Bayesian Inference and Maximum Likelihood analysis (Fig. 1). The genetic distance (uncorrected p-distance) between the specimens from China and the specimens (including type specimens) of *L. nguyenorum* from Vietnam was 1.1% (Table 2). Although morphological characters of

Table 1. Sequences used for phylogenetic analysis in this study.

Taxon	Voucher no.	Locality	GenBank no.
Limnonectes bannaensis	KIZ 011793	China, Yunnan, Xishuangbanna, Mengla	KU599849
Limnonectes cintalubang	KUHE 47859	Malaysia, Borneo, Sarawak, Serian	AB981409
Limnonectes fragilis	CIB 20081089	China, Hainan, Wuzhishan	AB526315
Limnonectes fujianensis	CIB ZJ 200806223	China, Jiangxi, Zixi	AB526311
Limnonectes isanensis	KUHE 19284	Thailand, Loei, PhuLuang	AB526314
Limnonectes isanensis	KUHE 19320	Thailand, Loei, PhuLuang	AB558955
Limnonectes jarujini	KUHE 19514	Thailand, Kanchanaburi, Sangkhla Buri	AB558940
Limnonectes jarujini	KUHE 19690	Thailand, SuratThani, Khlong Saeng	AB558950
Limnonectes longchuanensis	KIZ048424	China, Yunnan, Dehong, Longchuan	KU599867
Limnonectes longchuanensis	KIZ048527	Thailand, Loei, PhuLuang	KU599869
Limnonectes kuhlii	GMU unnumbered	Indonesia, Java, Purwerojo	AB526316
Limnonectes megastomias	KU 307760	Thailand, Nakon Ratchasima	HM067201
Limnonectes megastomias	FMNH 266221	Thailand, Sa Kaew, Pang Si Da	HM067184
Limnonectes namiyei	KUHE L0809191	Japan, Okinawa, Okinawajima	AB526309
Limnonectes quangninhensis	IEBR 3970	Vietnam, Quang Ninh, Hai Ha	KY595928
Limnonectes selatan	KUHE54079	Malaysia, Genting, Pahang	AB981384
Limnonectes taylori	KUHE 19101	Thailand, Chiang Mai, Doi Inthanon	AB558929
Limnonectes taylori	KUHE 19868	Thailand, Chiang Mai, Tha Ton	AB981390
Limnonectes utara	KUHE54064	Malaysia, Larut, Perak	AB981377
Limnonectes nguyenorum	VNMN A.2015.1	Vietnam, Ha Giang, Vi Xuyen, Cao Bo	HM067250
Limnonectes nguyenorum	IEBR A.2015.3	Vietnam, Ha Giang, Vi Xuyen, Cao Bo	HM067258
Limnonectes nguyenorum	AMNH A-163944	Vietnam, Ha Giang, Vi Xuyen, Cao Bo	HM067267
Limnonectes nguyenorum	VNMN A.2015.3	Vietnam, Ha Giang, Vi Xuyen, Cao Bo	HM067252
Limnonectes nguyenorum	IEBR 3967	Vietnam, Vinh Phuc, Phuc Yen	KY626298
Limnonectes nguyenorum	IEBR 3968	Vietnam, Vinh Phuc, Phuc Yen	KY626299
Limnonectes nguyenorum	HLNP 2018.003	Vietnam, Lao Cai, Sapa, Fansipan	MK951684
Limnonectes nguyenorum	KIZ20193243	China, Yunnan, Honghe, Gejiu	OL904956
Limnonectes nguyenorum	KIZ20193244	China, Yunnan, Honghe, Gejiu	OL904957
Limnonectes nguyenorum	KIZ20193245	China, Yunnan, Honghe, Gejiu	OL904958
Limnonectes nguyenorum	KIZ20193246	China, Yunnan, Honghe, Gejiu	OL904959
Limnonectes nguyenorum	KIZ20193247	China, Yunnan, Honghe, Gejiu	OL904960
Fejervarya limnocharis	AMNH A-161230	Vietnam, Nghe An, Con Cuong, Pu Mat	AY843588
Fejervarya iskandari	UI unnumbered	Indonesia, Java, Banyuwangi	AB526324

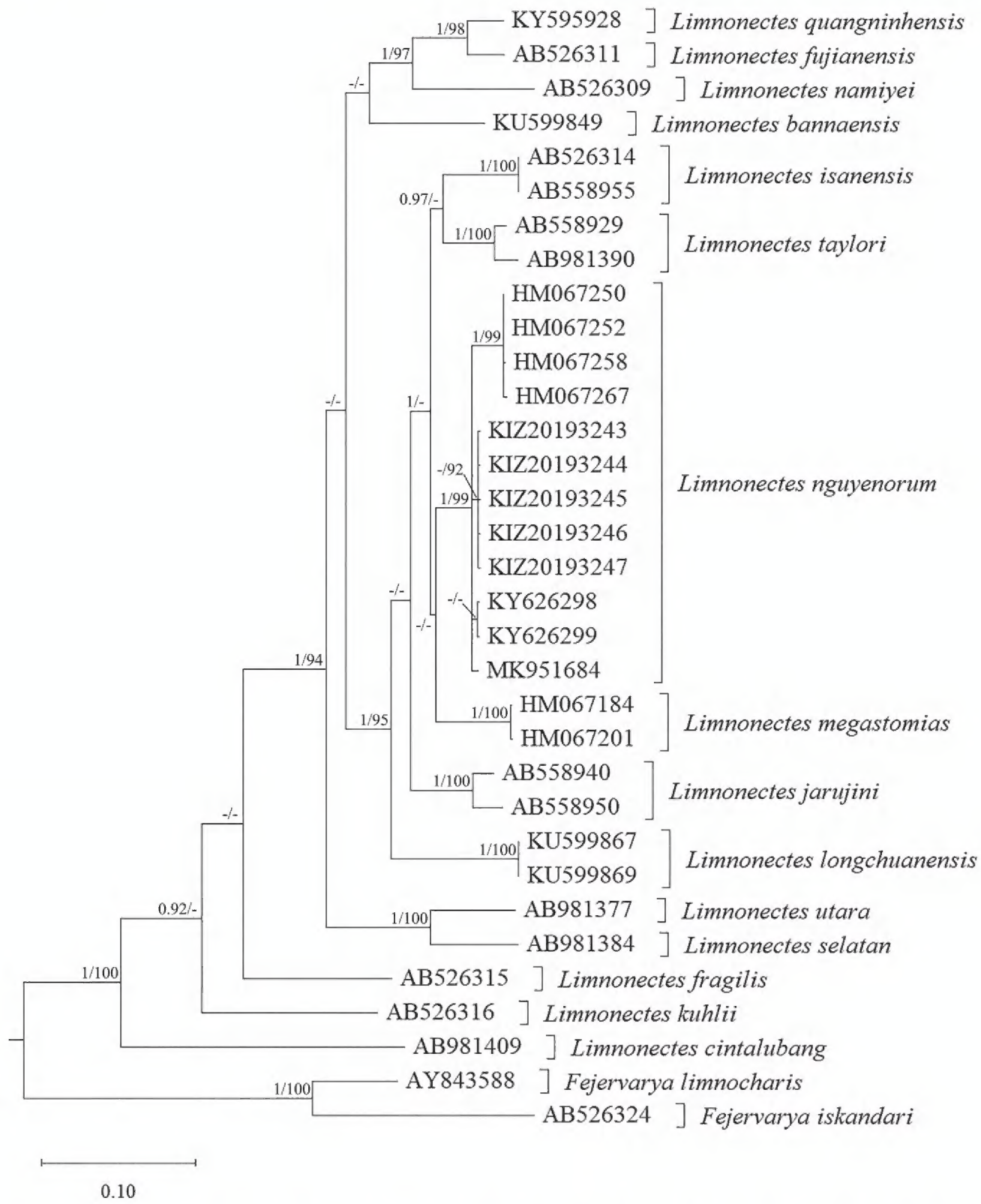

Figure 1. Bayesian Inference tree based on 900 bp 16S rRNA sequences. Numbers before slashes indicate Bayesian posterior probabilities (> 0.9 remain) and numbers after slashes indicate ultrafast bootstrap support for Maximum Likelihood analyses (> 90 remain).

Table 2. Genetic uncorrected p-distances (%) based on 900 bp 16S rRNA sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 *Limnonectes bannaensis*															
2 *Limnonectes cintalubang*	16.3														
3 *Limnonectes fragilis*	12.5	16.2													
4 *Limnonectes fujianensis*	10.3	17.4	12.6												
5 *Limnonectes isanensis*	12.0	17.0	13.3	11.9											
6 *Limnonectes jarujini*	11.6	17.5	14.6	11.8	8.8										
7 *Limnonectes kuhlii*	14.5	16.8	13.1	14.7	12.7	14.9									
8 *Limnonectes longchuanensis*	11.0	18.1	14.8	11.2	10.1	9.3	15.1								
9 *Limnonectes megastomias*	10.9	18.0	12.5	12.3	8.0	8.7	13.8	10.2							
10 *Limnonectes namiyei*	10.4	16.8	14.3	9.7	13.2	11.7	14.9	12.2	12.8						
11 *Limnonectes nguyenorum* (China)	6.6	13.9	9.2	8.0	4.5	4.5	9.6	5.6	4.3	7.6					
12 *Limnonectes nguyenorum* (Vietnam)	9.0	16.0	12.0	10.0	6.5	6.1	13.0	7.3	6.0	10.0	1.1				
13 *Limnonectes quangninhensis*	5.8	12.7	7.4	2.9	9.9	8.6	9.7	10.3	9.3	6.1	8.0	8.7			
14 *Limnonectes selatan*	14.3	16.4	13.9	12.1	12.7	13.4	15.2	13.3	13.9	13.1	10.3	12.0	9.2		
15 *Limnonectes taylori*	11.2	17.4	13.7	10.4	7.4	7.7	14.6	10.6	7.6	12.1	4.8	6.4	9.3	13.2	
16 *Limnonectes utara*	13.2	17.3	14.2	12.3	13.1	13.0	15.2	13.9	13.6	13.7	9.6	11.8	9.2	8.2	12.3

the specimens from China showed slight variations (smaller body size and nuptial pad present only on first finger) from the original description (McLeod et al. 2015) of *L. nguyenorum*, they agree with the subsequent descriptions (Pham et al. 2016; Le and Do 2019; Nguyen et al. 2019; Pham et al. 2019) of *L. nguyenorum* from Vietnam. Therefore, we consider that the specimens from Daweishan Nature Reserve, southern Yunnan, China, belong to *L. nguyenorum*.

Taxonomic account

Limnonectes nguyenorum McLeod, Kurlbaum & Hoang, 2015

Figs 2–3

Specimens examined. KIZ20193243–KIZ20193245, three adult males, and KIZ20193246–KIZ20193247, two adult females, all collected on 24 March 2019 from Daweishan Nature Reserve, Manhao Town, Gejiu City, Honghe Prefecture, Yunnan Province, China (23°1'21"N, 103°23'52"E; at an elevation of 500 m).

Morphological description. Morphometric and meristic data are presented in Table 3. Body size small (SVL 36.5–38.5 mm in males, 35.4–35.5 mm in females); head large (HL 42–44% SVL in males, 36–39% in females); head slightly wider than long in males (HL 92–94% HW), head length almost equal to width in females (HL 97–101% HW); canthus rostralis indistinct and rounded, lores flat; supratympanic fold distinct; tympanum not visible in males and indistinct in females; odontoid processes in males robust with rounded tips, angled posteriorly, odontoid processes present but very short in females.

Limbs robust, relatively short. Finger tips rounded, relative length of fingers usually III>IV>II>I, rarely III>IV>I>II; no webbing on fingers; nuptial pad on finger I present in males. Toe tips rounded, relative length of toes IV>III>V>II>I. Tibia short (TBL 43–48% SVL), slightly shorter than femur. Full webbing between toes, inner metatarsal tubercle oval, a skin ridge from inter metatarsal to tarsus present.

Skin on top of the head and venter smooth, skin on dorsum, flanks, and dorsal surface of forelimbs with sparse tubercles; precloacal area and dorsal surface of hindlimbs covered with heterogeneous dense tubercles.

Coloration. Head yellow–brown with brownish black bar on posterior interorbital region, yellowish white bar extending from nares to insertion of arm, upper lip with distinct white spots and dark brown bars, dorsum and dorsal surfaces of limbs greyish yellow–brown, supratympanic fold brownish black, lateral sides greyish, ventral surface of throat and chest white with black marbling, ventral surface of limbs and abdomen white, dorsal surface of thighs and tibias with brownish black bars; iris reddish brown in upper half and greyish white in lower half.

Extended diagnosis. Based on the newly collected specimens from China and the descriptions (McLeod et al. 2015; Pham et al. 2016; Le and Do 2019; Nguyen et al. 2019; Pham et al. 2019) of *L. nguyenorum* from Vietnam, we extend the diagnosis of this species: (1) adult male SVL 36.5–47.3 mm, adult female SVL 35.4–47.1 mm; (2) males with nuptial pads on first finger or first and second fingers; (3) males with slightly enlarged heads (HL 37–46% of SVL in males; 36–43% in females); (4) head slightly longer than wide or slightly wider than long in males(HL 92–112% HW), head slightly longer than wide or length almost equal to width

Table 3. Measurements (in mm) and proportions of the specimens of *Limnonectes nguyenorum* from Yunnan, China. For abbreviations see Materials and methods.

	KIZ20193243	KIZ20193244	KIZ20193245	KIZ20193246	KIZ20193247
	male	male	male	female	female
SVL	37.1	36.5	38.5	35.5	35.4
ED	4.7	4.5	5.0	4.4	4.7
EN	3.4	3.4	3.2	2.9	3.0
RL	6.3	6.2	6.7	5.3	5.7
FEL	18.3	18.5	19.2	16.9	17.9
FOL	25.6	24.9	26.3	23.2	24.9
HL	15.6	15.5	17.1	12.9	13.8
HW	16.8	16.7	18.1	13.3	13.7
IN	3.6	3.8	3.9	3.2	3.1
IO	3.3	2.9	3.5	2.6	2.6
LAL	7.1	7.3	7.9	6.2	7.2
PAL	9.2	9.3	10.4	8.3	8.8
RFL	III>IV>II>I	III>IV>II>I	III>IV>II>I	III>IV>II>I	III>IV>I>II
RTL	IV>III>V>II>I	IV>III>V>II>I	IV>III>V>II>I	IV>III>V>II>I	IV>III>V>II>I
TBL	17.1	16.8	18.1	15.1	16.9
TD	2.2	2.3	2.4	2.3	2.3
UEW	2.9	2.5	2.8	2.8	2.8
OH	2.7	2.2	2.8	1.4	1.5
PAL/LAL	1.26	1.22	1.28	1.30	1.19
PAL/SVL	0.25	0.25	0.27	0.23	0.25
ED/HL	0.30	0.29	0.29	0.34	0.34
HL/HW	0.92	0.93	0.94	0.97	1.01
HL/SVL	0.42	0.42	0.44	0.36	0.39
TBL/SVL	0.46	0.46	0.47	0.43	0.48
FEL/SVL	0.49	0.51	0.50	0.48	0.51
LAL/SVL	0.19	0.20	0.21	0.17	0.20
FOL/SVL	0.69	0.68	0.68	0.65	0.70

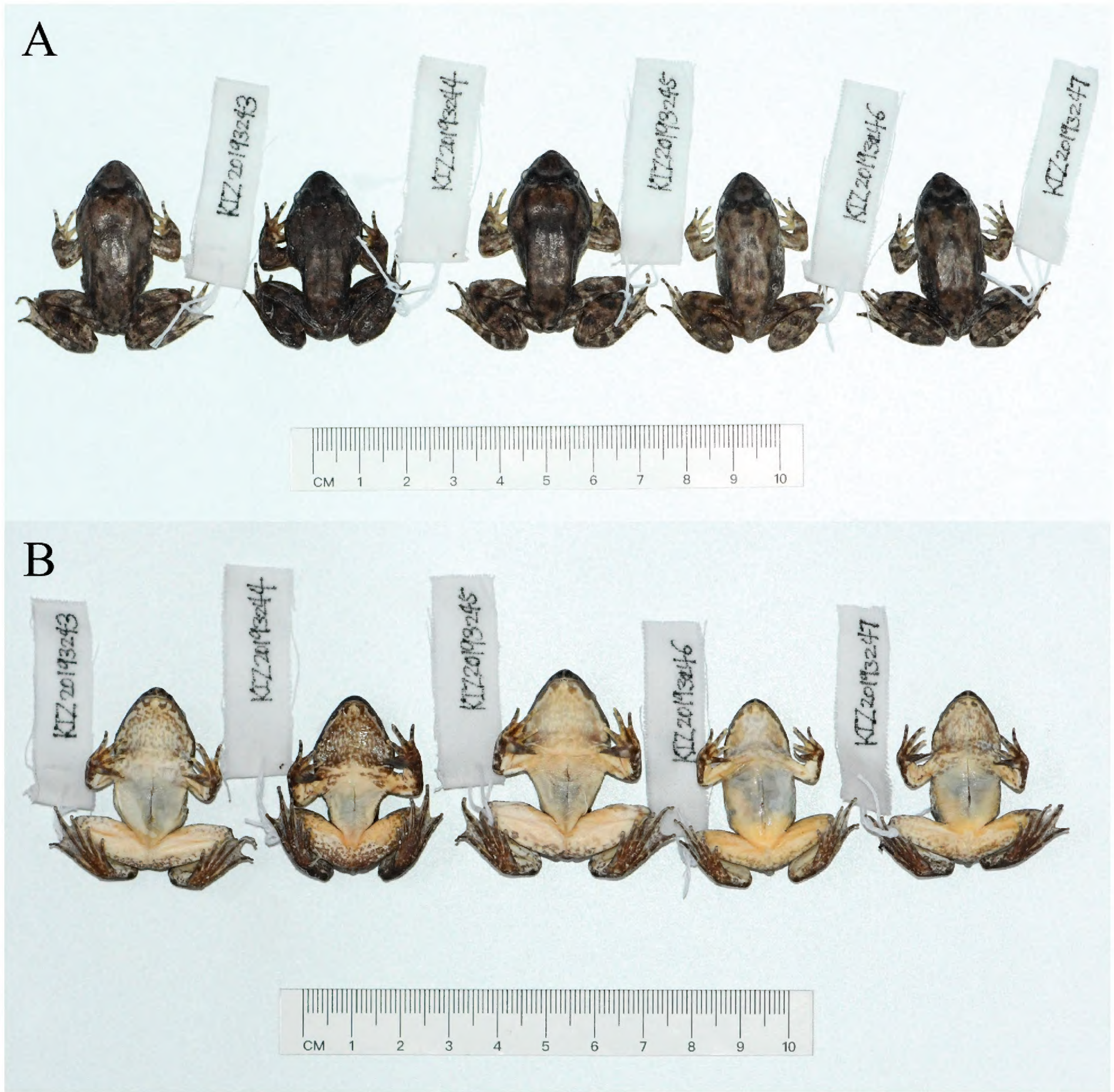

Figure 2. Specimens of *Limnonectes nguyenorum* from Daweishan Natural Reserve, southern Yunnan, China, in preservative. **A.** Dorsal view; **B.** Ventral view.

in females (HL 97–116% HW); (5) canthus rostralis indistinct and rounded, lores flat; (6) supratympanic fold distinct or indistinct; (7) tympanum indistinct (or not visible); (8) white or yellow bar extending from nares to insertion of arm, upper lip with distinct white spots and dark brown bars; (9) skin on top of head and venter smooth, skin on dorsum and flanks very feebly crenulate; (10) pericloacal area, and dorsal surfaces of shank and foot covered with heterogeneous tubercles; (11) toes fully webbed; (12) relative finger length when adpressed: III>IV>II>I or III>IV>I>II.

Ecological notes. The specimens were found at night in the artificial drainage ditch along a road; there are primary forests on both sides of the road, and there was shallow water flowing in the ditch. No eggs or tadpoles were found.

Discussion

Limnonectes nguyenorum was known previously only from northern Vietnam (Frost 2021). This is the first record of *L. nguyenorum* from China and thus from outside of Vietnam. The new locality in China is approximately 150 km away from the type locality in Vietnam (Fig. 4). Our work brings the total species number of the genus *Limnonectes* in China to seven.

Two new species of amphibians and reptiles have been recently described from Daweishan Nature Reserve (Liu et al. 2021; Zhang et al. 2021) and the diversity of amphibians and reptiles in this area is still underestimated. Further study is necessary to document the true amphibian and reptile diversity of Daweishan Nature Reserve.

Figure 3. *Limnonectes nguyenorum* from Daweishan Natural Reserve, southern Yunnan, China, in life. **A.** Dorsal view of the adult male (KIZ20193245); **B.** Ventral view of the adult male (KIZ20193245); **C.** Dorsal view of the adult female (KIZ20193246); **D.** Ventral view of the adult female (KIZ20193246).

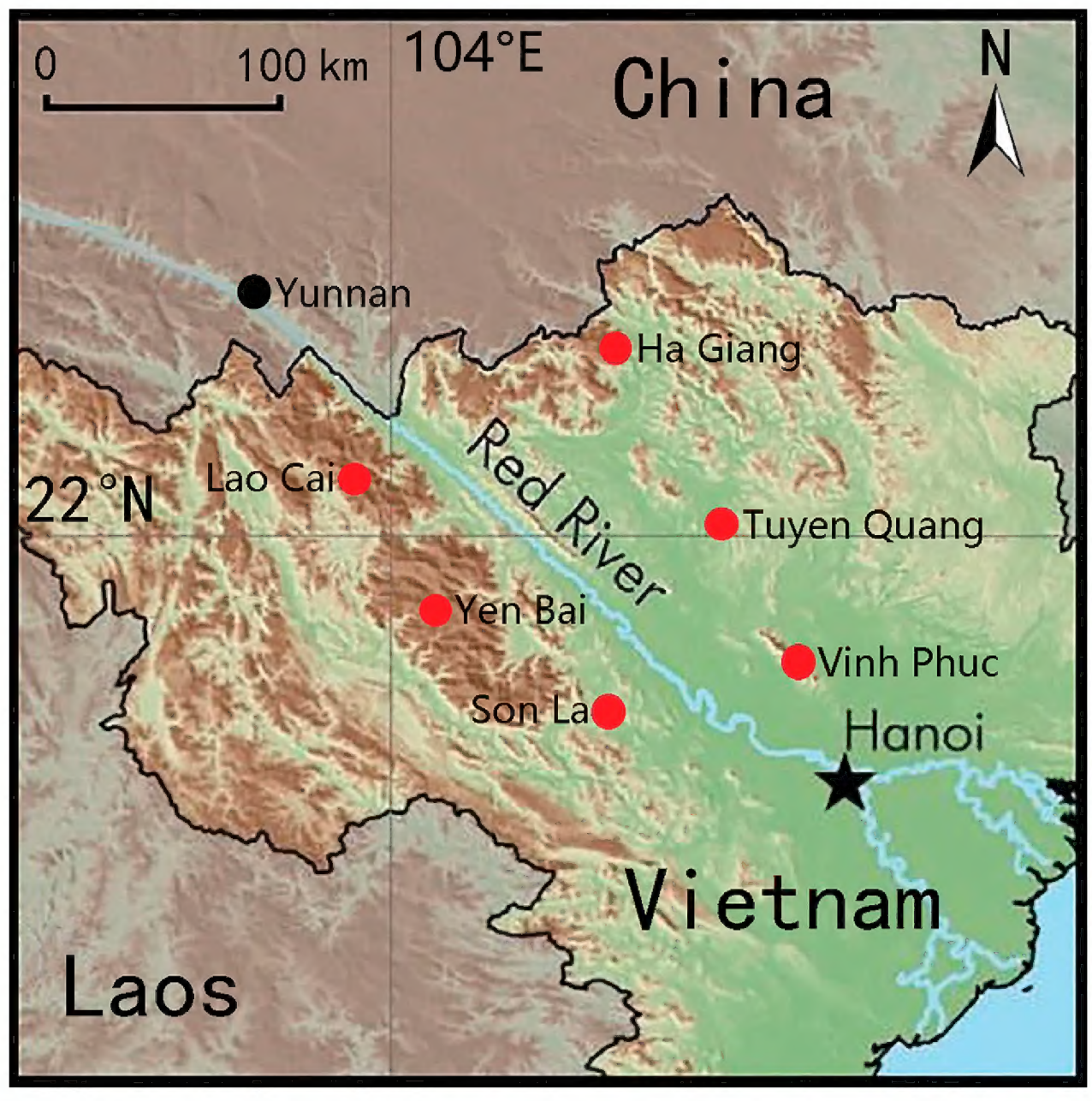

Figure 4. Map showing the known distribution of *Limnonectes nguyenorum* in Vietnam (red dots) and in China (black dot).

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